*Short communication***Protein metabolism in glucagonoma****R. Barazzoni, M. Zanetti, A. Tiengo, P. Tessari**

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Summary Although protein wasting and reduced amino acid concentrations are common findings in glucagonoma patients, the mechanisms underlying these alterations are unclear. Therefore, we studied basal postabsorptive leucine, phenylalanine and tyrosine turnover following L-[D₃]-Leucine, L-[D₅]-Phenylalanine and L-[D₂]-Tyrosine i.v. infusions in one male and one female patient with glucagonoma, compared with healthy control volunteers. Plasma amino acid concentrations were reduced (–40 to 80 %, $\delta > 2$ SD vs control subjects) in both patients. Plasma leucine, phenylalanine and tyrosine rates of appearance in patients with glucagonoma were similar to values in the control subjects, except leucine rate of appearance in the female patient with glucagonoma (+ ≈ 30 %, $\delta > 2$ SD). In contrast, the intracellular leucine rate of appearance, reflecting protein degradation, was considerably increased in both patients (+ 60–80 %, $\delta > 2$ SD). Phenylalanine

hydroxylation was moderately higher only in the male patient with glucagonoma (+ ≈ 30 %, $\delta > 2$ SD). Leucine, phenylalanine and tyrosine clearances (+ 100–300 %), as well as phenylalanine hydroxylative clearance (+ 75–100 %) were also increased in the patients. In conclusion, whole-body protein breakdown is enhanced in patients with glucagonoma compared with healthy control subjects. Phenylalanine hydroxylative clearance is also higher. Reduced plasma amino acid concentrations are probably due, at least in part, to their increased clearance. These alterations could contribute to the determination of the catabolic state of the glucagonoma syndrome. [Diabetologia (1999) 42: 326–329]

Keywords Glucagonoma, glucagon, rate of appearance, protein breakdown, phenylalanine hydroxylation.

Patients with glucagon-producing pancreatic tumours exhibit profound metabolic disturbances, leading to the typical glucagonoma syndrome [1]. In particular, weight loss, muscle mass reduction and hypoaminoacidaemia suggest a sustained protein catabolism probably due, at least in part, to glucagon excess. Indeed, acute hyperglucagonaemia in healthy humans reduced plasma amino acid concentration [2] and en-

hanced essential amino acid catabolism [3, 4], although no effects on whole-body proteolysis were detected in the presence of basal insulin concentrations [3, 4]. Rather surprisingly, when leucine and protein kinetics were studied in a patient with glucagonoma, only a slight increase of leucine oxidation was reported, and protein breakdown was normal [5]. This patient had been treated, however, with i.v. octreotide and amino acids, which might have influenced protein turnover during the study [6]. Therefore, the mechanisms leading to protein wasting in glucagonoma are still not completely understood. To investigate this issue, postabsorptive leucine, phenylalanine and tyrosine kinetics were studied in two glucagonoma patients prior to treatment, and compared with normal control subjects values.

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Abbreviations: Ra; Rate of appearance.

Subjects and methods

Patients. One man (age: 28 years, BMI: 22.1 kg/m²) and one woman (age: 59 years, BMI: 19.1 kg/m²) with glucagonoma were studied. The man presented with weight loss (25 kg over 12 months) and skin rash on the abdomen and the back; he was otherwise in good general health and free of abdominal symptoms. Fasting plasma glucose and insulin concentrations were mildly raised (Table 1), while glucagon levels were constantly above 500 ng/l. The woman was admitted for weight loss (32 kg over 30 months), skin rash on the abdomen, buttocks and legs, and a recently developed diabetes mellitus. She was in poor general and nutritional health, although she complained of no abdominal symptoms and followed a 2000 calories-a-day diet. The glycaemic control was poor (HbA_{1c} = 9.6%), but not sufficient to justify the clinical picture, and greatly increased (> 4.000 ng/l) plasma glucagon concentrations were detected.

In both patients, ultrasound, computerised tomography and ¹¹¹IN-octreotide scintigraphy showed nodular lesions in the pancreas and liver. Protein turnover studies were done before the start of antitumour therapy. The man was not taking any medications, but the woman received insulin injections at meal times plus parenteral glucose, insulin, lipids and amino acids. Amino acids had been given for three days (6.25 g N/day), and were withdrawn three days before the study. All data were compared with those obtained in healthy male volunteers (age: 29 ± 3 years, BMI: 23 ± 1 kg/m²).

Isotopes. We purchased L-[5,5,5-²H]-leucine (D₃-leucine), L-[ring-²H₅]-phenylalanine (D₅-phenylalanine) and L-[3,3-²H]-tyrosine (D₂-tyrosine) from Tracer Technologies Inc. (Somer-ville, Mass., USA), and L-[ring-²H]-tyrosine (D₄-tyrosine) from Euriso-Top (Gif-Sur-Yvette, France). All isotopes were proven sterile and pyrogen-free before use.

Experimental protocol. In the absence of an ethics committee at our University, the study protocol was approved by the dean of the Faculty of Medicine and the head of the department. All participants were informed about the aims and potential risks of the study and provided a written consent. At about 0730 hours, with subjects under postabsorptive conditions, polyethylene catheters were placed in an antecubital vein of the right arm for isotope infusion and, in a retrograde fashion, in a wrist vein of the contralateral arm for arterialized blood sampling. At 0800 hours (0 min), a primed (60-fold rate of infusion per min⁻¹) continuous infusion of D₃-leucine, D₅-phenylalanine and D₂-tyrosine was started. A priming dose of D₄-tyrosine (≈ 0.4 μmol/kg) was also given, to prime the phenylalanine-derived tyrosine pool [7]. Rates of isotope infusion (μmol · kg⁻¹ · min⁻¹) were approximately 0.05 (D₃-leucine), approximately 0.05 (D₅-phenylalanine) and approximately 0.02 (D₂-tyrosine) in the woman and control subjects, and 0.078, 0.05 and 0.03, respectively in the man. Blood samples were collected every 10 min for the last 30 min of the study (150 min-180 min).

Analytical methods. We determined D₃-leucine, D₃-α-ketoisocaproate (KIC), D₅-phenylalanine, D₂-tyrosine and D₄-tyrosine plasma isotopic mole percent enrichments (MPE) by gas chromatography-mass spectrometry (GC-MS) as tert-butyl-dimethyl-silyl derivatives [4]. The monitored masses (m/z) were: 203/200 for M + 3/M + 0 leucine; 304/301 for M + 3/M + 0 α-ketoisocaproate; 239/234 for M + 5/M + 0 phenylalanine; 470/468/466 for M + 4/M + 2/M + 0 tyrosine. Plasma amino acids were measured by Ion Exchange Liquid Chroma-

tography with a Beckman amino acid analyser (Beckman Instruments, Palo Alto, Calif., USA). Blood glucose was measured with a Beckman glucose analyser 2. Hormone concentrations were measured by radioimmunoassay [4].

Calculations and statistical analysis. Leucine, phenylalanine and tyrosine plasma rates of appearance (Ra, μmol · kg⁻¹ · min⁻¹) were calculated by conventional formula [4]. Leucine intracellular rate of appearance (Ra_{ic}), reflecting protein breakdown, was calculated using the reciprocal pool model [4]. Phenylalanine hydroxylation was directly measured [7]. Plasma amino acid clearances (ml · kg⁻¹ · min⁻¹) were calculated by dividing their plasma Ra, which equals the rate of disappearance at steady state, over the corresponding plasma concentration (μmol/l). Statistical analysis was limited to calculation of mean values and standard deviation (SD). Differences (δ) between patients, and control subjects, mean values greater than 2 SD are indicated.

Results

Substrates, hormones (Table 1). Plasma glucagon concentrations were 4764 and 591 ng/l for the woman and the man, respectively with average control values of 85 ± 25 ng/l. Plasma insulin and glucose concentrations were higher in patients, but C-peptide concentrations were similar in both groups. Plasma amino acid concentrations were considerably decreased in both patients. Selected values are reported in Table 1.

Leucine, phenylalanine and tyrosine kinetics (Table 2). In the last 30 min of the study MPEs of all isotopes were at steady state (data not shown). Leucine plasma Ra was within normal values in the man but it was approximately 30% higher in the woman (δ > 2 SD). Phenylalanine Ra was similar in the patients and control subjects but tyrosine Ra was slightly lower in the patients. In contrast, leucine Ra_{ic}, reflecting whole-body protein breakdown, was 60–80% higher in both patients than in the control subjects (δ > 2 SD).

Phenylalanine hydroxylation was similar in the woman, and about 30% increased in the man (δ > 2 SD) compared with the control subjects. Leucine, phenylalanine and tyrosine clearances were two to four-fold greater in the patients (δ > 2 SD). Since phenylalanine plasma concentration ([Phe]) and hydroxylation were correlated in the control subjects ($r^2 = 0.32$, $p = 0.04$), phenylalanine hydroxylative clearance (Phe Hy Cl, ml · kg⁻¹ · min⁻¹) was calculated as follows:

$$\text{Phe Hy Cl} = \frac{\text{Phe Hy}}{[\text{Phe}]} \times 100$$

Phe Hy Cl, an index of phenylalanine hydroxylase activity independent of phenylalanine concentrations, was 75–100% higher in patients compared with the control subjects (δ > 2 SD).

Table 1. Glucagon (ng/ml), insulin (pmol/l), c-peptide (nmol/l), glucose (mmol/l) and selected amino acid ($\mu\text{mol/l}$) plasma concentrations in patients and in control subjects

	Male patient	Female patient	Control subjects
Glucagon	591 ^a	4764 ^a	85 $\pm$ 25
Insulin	132 ^a	102	60 $\pm$ 24
C-peptide	0.46	0.40	0.40 $\pm$ 0.07
Glucose	6.9 ^a	10.6 ^a	4.5 $\pm$ 0.5
Alanine	69 ^a	65 ^a	273 $\pm$ 78
Serine	68 ^a	49 ^a	104 $\pm$ 18
Glycine	99 ^a	77 ^a	173 $\pm$ 36
Proline	36 ^a	20 ^a	235 $\pm$ 93
Glutamine	94 ^a	44 ^a	175 $\pm$ 28
Lysine	72 ^a	49 ^a	144 $\pm$ 16
Arginine	19 ^a	23 ^a	73 $\pm$ 7
Methionine	3 ^a	5 ^a	14 $\pm$ 3
Leucine	52 ^a	48 ^a	129 $\pm$ 18
Valine	81 ^a	70 ^a	200 $\pm$ 42
Isoleucine	32 ^a	28 ^a	53 $\pm$ 8
Phenylalanine	35	32	46 $\pm$ 8
Tyrosine	15 ^a	16 ^a	47 $\pm$ 10

Control subject data are Means $\pm$ SD; ^a: > or < 2 SD vs N

Discussion

Our study shows that whole-body protein breakdown is increased in glucagonoma patients, and this alteration could have a pivotal role in the loss of lean body mass commonly observed in this syndrome [1]. Previous reports on the effects of acute hyperglucagonaemia in healthy humans did not find any significant stimulation of postabsorptive whole-body proteolysis [3, 4]. Glucagon concentrations were, however, lower than in the current study and hyperglucagonaemia was maintained for a relatively short time. Indeed, the present results suggest that enhanced protein breakdown could be a long-term effect of very high glucagon concentrations. Prolonged glucagon-induced hypoaminoacidaemia could also contribute to the increased proteolysis, since amino acids promote protein deposition and inhibit protein breakdown [6].

Skeletal muscle mass, representing the bulk of body proteins, was reduced in the patients based on clinical observation, as well as on plasma creatinine concentration [man = 53, woman = 26, control subjects ($n = 9$) = 83 $\pm$ 12 $\mu\text{mol/l}$, $\delta > 2$ SD]. This observation emphasizes the increment of protein breakdown, which would be quantitatively even greater if normalized per body nitrogen content. Whereas muscle wasting is one of the hallmarks of glucagonoma [1], in previous reports glucagon had minor short-term effects on in vivo skeletal muscle protein metabolism [8]. Again, prolonged hyperglucagonaemia and hypoaminoacidaemia might specifically enhance muscle proteolysis. Furthermore, it cannot be excluded that other unidentified tumour-related factors could stimulate regional protein breakdown. Finally, other tissues, including the splanchnic region, are likely to contribute to the increased whole-body proteolysis, as indirectly suggested by the higher rates of

Table 2. Leucine (Leu) intracellular rate of appearance ($R_{a_{ic}}$, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), Leu, phenylalanine (Phe) and tyrosine (Tyr) plasma rates of appearance (R_a , $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), Phe hydroxylation (Phe Hy, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), Leu, Phe and Tyr clearance (Cl, $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and Phe hydroxylative clearance (Phe Hy Cl, $\text{ml/kg} \cdot \text{min}$) in patients and in control subjects

	Male patient	Female patient	Control subjects
Leu $R_{a_{ic}}$	3.32 ^a	3.02 ^a	1.94 $\pm$ 0.24
Leu R_a	1.37	1.87 ^a	1.47 $\pm$ 0.15
Phe R_a	0.67	0.76	0.65 $\pm$ 0.14
Tyr R_a	0.38	0.39	0.51 $\pm$ 0.11
Phe Hy	0.115 ^a	0.084	0.088 $\pm$ 0.01
Leu Cl	2.98 ^a	3.9 ^a	1.1 $\pm$ 0.18
Phe Cl	1.98 ^a	2.37 ^a	1.21 $\pm$ 0.23
Tyr Cl	2.74 ^a	2.46 ^a	1.01 $\pm$ 0.34
Phe Hy Cl	0.34 ^a	0.26 ^a	0.16 $\pm$ 0.03

Control subject data are Means $\pm$ SD; ^a: > 2 SD vs N

protein breakdown in the presence of reduced muscle mass in the patients.

At variance with our results, in the only other available study of leucine turnover in a patient with glucagonoma, normal rates of plasma and intracellular leucine appearance were reported [5]. This patient had, however, been treated with i.v. octreotide and amino acids until a few hours before the study [5]. In particular, the exogenous amino acid infusion might have directly suppressed protein breakdown [6], resulting in minor or no alterations of leucine turnover.

The increased leucine, phenylalanine and tyrosine clearances are likely to contribute to the reduced plasma concentrations of these amino acids. Enhanced clearances, as well as the higher ratio of intracellular to plasma leucine rates of appearance, also suggest a relative intracellular amino acid accumulation in glucagonoma. In a previous report the reduction of amino acid concentrations was less pronounced in the liver and skeletal muscle than in plasma in a glucagonoma patient [9], and relatively higher intracellular amino acids are in keeping with the known glucagon-dependent stimulation of amino acid inward transport across the cell membrane [4].

Phenylalanine hydroxylation, the first liver-located step of phenylalanine irreversible catabolism by phenylalanine hydroxylase [4, 7], was normal or moderately increased in the patients. When hydroxylation was normalized for plasma phenylalanine concentration and expressed as hydroxylative clearance, it was, however, about twofold greater than normal in both patients, in agreement with the stimulatory effect of glucagon on phenylalanine hydroxylase activity in vitro [10]. Since phenylalanine is an indispensable amino acid whose irreversible catabolism reflects net protein loss [4], this finding might represent a further mechanism contributing to protein wasting in glucagonoma.

In conclusion, the present data show that whole-body protein breakdown is increased in patients with

glucagonoma compared with normal control subjects. Leucine, phenylalanine and tyrosine clearances, as well as phenylalanine hydroxylative clearance, are also enhanced in the patients. These combined alterations could have an important role in determining the protein-catabolic state and the hypoaminoacidaemia observed in conditions of long-term glucagon excess.

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